Developing ChemFin™, a Miniature Biogeochemical Sensor Payload for Gliders, Profilers, and other AUVs

PI: Alfred K. Hanson
SubChem Systems, Inc.
65 Pier Road
Narragansett, RI 02882
phone: (401) 783-4744 Ext. 102 fax: (401) 783-4744 e-mail: hanson@subchem.com

Grant Number: N000140811026
http://www.subchem.com

Co-PI: Anne Kusterbeck
Naval Research Laboratory
4555 Overlook Ave., SW
Washington, DC 20375
phone: (202) 404-6042 e-mail: akusterbeck@cbmse.nrl.navy.mil

Grant Number: N0001409W20359

LONG-TERM GOALS

The first goal of this project involves the further development and transition of ChemFIN™, a prototype autonomous profiling sensor for chemicals and biomolecules, into a commercial product that can be readily deployed on fixed or mobile ocean observation platforms such as coastal gliders, profiling moorings, and propeller driven unmanned underwater vehicles (UUVs). The second goal of this project is to integrate a flow immunosensor technology (i.e. biosensor), developed by researchers at the Naval Research Laboratory, into ChemFIN for the detection of biomolecules of interest, such as specific biotoxins (i.e saxitoxin) that are released during harmful algal blooms (HABs). ChemFIN is being developed for sustained, autonomous ocean observations of specific chemical and biochemical distributions and spatial and temporal variability. ChemFIN is an evolving compact sensor payload, utilizing microfluidics, and is particularly designed for "low-power" underway measurements on gliders, propeller-driven autonomous underwater vehicles (AUVs) and autonomous profilers.

OBJECTIVES

The first objective is to use recent advances in micro-fluidics and optical detectors to improve the ChemFIN sensor. The technical improvements involve reducing sample flow rates and volumes and thus reagent and power consumption, extending the length of field deployments by developing new technologies to suppress bio-fouling, increasing the ease of use by simplifying operation, pre-packaging reagents and thoroughly documenting the performance by conducting demonstration experiments in coastal waters. The second objective is to adapt and integrate the flow immunosensor

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
30 SEP 2009	**Annual**	**00-00-2009 to 00-00-2009**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Developing ChemFinTM, A Miniature Biogeochemical Sensor Payload For Gliders, Profilers, And Other AUVs	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **SubChem Systems, Inc.,65 Pier Road,Narragansett,RI,02882**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES
Code 1 only

14. ABSTRACT

The first goal of this project involves the further development and transition of ChemFINTM, a prototype autonomous profiling sensor for chemicals and biomolecules, into a commercial product that can be readily deployed on fixed or mobile ocean observation platforms such as coastal gliders, profiling moorings, and propeller driven unmanned underwater vehicles (UUVs). The second goal of this project is to integrate a flow immunosensor technology (i.e. biosensor), developed by researchers at the Naval Research Laboratory, into ChemFIN for the detection of biomolecules of interest, such as specific biotoxins (i.e saxitoxin) that are released during harmful algal blooms (HABs). ChemFIN is being developed for sustained, autonomous ocean observations of specific chemical and biochemical distributions and spatial and temporal variability. ChemFIN is an evolving compact sensor payload, utilizing microfluidics, and is particularly designed for ?low-power? underway measurements on gliders, propeller-driven autonomous underwater vehicles (AUVs) and autonomous profilers.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**7**	

analytical technology, developed by NRL researchers, into the MARCHEM and ChemFIN sensor payloads.

APPROACH AND WORKPLAN

These objectives are being achieved through a partnership between industry (Alfred Hanson, SubChem Systems, Inc.) and government (Anne Kusterbeck, NRL). The two partners have prior experience working together to develop and test new biological/chemical sensing and deployment systems. During this project, the industry partner will take the lead in developing and testing the commercial versions of the MarChem and ChemFIN sensor payloads while the government partner will take the lead in the development of alternative analytical technologies for the flow immunosensor application and assistance with the testing and performance evaluation.

WORK COMPLETED

Design and developmental testing was continued this year by SubChem Systems on a new submersible chemical analyzer. ChemFIN^{TM} (Figure 1) is a small independent sensor payload, utilizing microfluidics, and is particularly designed for "low-power" underway measurements on gliders, propeller-driven AUVs and autonomous profilers. SubChem engineers completed a very detailed design study for a "fourth generation" ChemFIN prototype. ChemFIN Prototype 4 is configured for the measurement of nitrates and is the first revision thus far that is a field deployable instrument (Figure 1). Laboratory experiments were conducted to evaluate the analytical capability for ChemFIN to measure low concentration levels of nitrate and nitrite.

Collaborative design work and info-exchange discussions between SubChem and NRL also continued on the integration of the NRL Flow Immunosensor technology into the MarChem Analyzer. NRL efforts focused this year on developing microfluidic coupons for high sample throughput in the immunosensor payload and producing anti-saxitoxin antibody assays. NRL continued their efforts to develop an immunoassay for saxitoxin to be incorporated into the biosensor payload. For these assays, antibodies will be produced both as standard monoclonal antibodies (mAb) and as single domain antibodies (sdA) isolated from llamas. Work also continued on MarChem sensor prototype testing, including incorporation of a next-generation optical detector. A new high-sensitivity, laser-based optical fluorescence detector for the detection of CY-5 fluorophore was designed, fabricated and incorporated into the MarChem Analyzer for testing and evaluation. New microfluidic coupons (Figure 2) were also designed and fabricated by NRL scientists and provided to SubChem for incorporation in the MarChem Immunosensor Payload (Figure 4). SubChem designed and fabricated a special face sealing coupon holder on the end-cap of the instrument. NRL scientists and SubChem Systems engineers and chemist spent a week working together in September 09 testing the instrument.

RESULTS

(A) ChemFIN Design Study. Based on the findings from testing previous ChemFIN prototypes, the improved fluidic system consists of a flow-through fluidic manifold with several external connections. Leading the system is a sample inlet, calibration standard addition, and two reagent inlets. A single sample pump resides at the end of the system to pull fluid through all the individual paths. The flow rates through the individual reagent and calibration inlets are metered with the use of narrow bore PEEK tubing. This prototype uses a custom sample pump. The wetted materials contained within the

pump are resilient enough to withstand the mixed chemicals at the end of the system. Included in the design is a thin film resistive heater which is adhered to one side of the micro-fluidic manifold and used to heat the entire manifold as well as the fluid contained within. The theory is that the fluid moves slow enough that the manifold temperature would translate to the fluid. The results from the power study provided the information required to allow the ChemFIN design to be as low power as possible.

(B) MarChem flow immunosensor payload development. The engineering design-path was further refined for the incorporation of the flow immunosensor technology into first the MarChem and eventually the ChemFIN analyzers. The testing and evaluation of a specialized, user-friendly, coupon holder, and multiple designs for ChemFIN's micro-fluidic manifold, provided results that allowed further advancement of the instrument. The new laser-based optical fluorescence detector for the flow immunosensor performed as well as or better than commercial laboratory fluorometers.

Improved Coupon Design - The disposable microfluidic coupons originally used in the biosensor provided limited capability for high throughput testing. The current assay response was limited by the 0.1 mL/min flow rate used for the prototype and the single channel path for sampling. To overcome these limitations, a new coupon was designed engineered and tested by NRL. The new coupon design (Figure 2) features a universal inlet/outlet with common reservoirs that feed the centrally located capture channels. The large circles at the corners are ¼ in dia. through holes used to demold the PMMA once embossed and to align the embossing chamber with the molding tool. The inner raised circles at the corners are dual purpose as well. They serve as alignment markers prior to thermal annealing and they provide markers for through holes that are later used to mount the coupon in the vessel. The 6 small circles within the triangular regions are support structures used to prevent collapse of the coverslip during thermal annealing. Finally, the inner microchannels are used to quantitate the analyte via displacement immunoassay.

(C) Saxitoxin Assay Development by NRL- In collaboration with Dr. Sherwood Hall, US FDA, conjugates were prepared and tested using polyclonal antibodies and initial antibodies produced in llamas, which are single domain antibodies rather than the classic double- chain IgGs.
Rabbit-Antisaxitoxin Assays - Fluid array competitive assays for saxitoxin were developed on the Luminex 100. Sets of microspheres were coated with the Saxitoxin-ovalbumin conjugates along with control proteins. These bead sets were incubated with either Bt-Rabbit anti-saxitoxin or Bt-Rabbit anti NeoSaxitoxin antibodies in the presence of various concentrations of Saxitoxin. After 30 min the unbound antibody was removed by filtration, and the microspheres were washed with buffer. SA-PE (5 ug/mL) was then added to tag the antibody bound to the microspheres. This amount is then quantified on the Luminex 100. As the amount of Saxitoxin increased, the binding by both rabbit polyclonals was inhibited. The limit of detection for Saxition in this assay is in the low ng/mL range.

Llama Anti-saxitoxin Production - Two llamas were immunized with saxitoxin-ovalbumin. A preimmune and post-immune sample of plasma were titered for the presence of anti-saxitoxin antibodies. Both animals responded to the antigen. The data is a direct binding assay to saxitoxin-ovalbumin coated luminex microspheres. Dilutions of plasma in a buffer solution were added to the microspheres; after washing by filtration, 1 ug/mL of Bt-goat anti-llama was added, washed again, then tagged with SA-PE 5 ug/ml, and measured by the Luminex 100. Llama IgG was purified from the immune plasma and tested to see if antibody was present which recognized the saxitoxin. A competitive assay was performed similar to those using the rabbit IgG. Like the rabbit IgG, the binding of the llama IgG to saxitoxin-ovalbumine coated microsphers was found to be inhibited by the

addition of free saxitoxin (Figure 3). The titer was not high enough to develop sensitive assays, but the results confirm the plan to develop single domain antibodies from these animals PBL's RNA.

IMPACT/APPLICATIONS

The oceanographic community does not currently have the capability to make routine and sustained biogeochemical measurements, *in situ* and autonomously, at the same space and time scales that are possible for temperature, salinity, oxygen, and chlorophyll fluorescence. In recent years, though, there has been significant progress in the development and application of reagent-based optical chemical sensors. The on-going research for this NOPP project is giving us the opportunity to further develop, improve and demonstrate these autonomous chemical sensing technologies. These efforts represent substantial advancements in the development of this technology and bring us much closer to a demonstrated capability for sustained, autonomous ocean observations of biogeochemical distributions and variability.

RELATED PROJECTS

SubChem Systems was recently awarded an FY09 Phase 1 SBIR Contract from NAVSEA (N6553809M0064) for Topic N091-46 "Compact, Lightweight Chemical Sensor for Underwater Explosive Ordnance (EOD) Application". WET Labs, Inc., SubChem Systems, Inc. and other partners also have a FY08 NOPP project "Long-term in situ chemical sensors for monitoring nutrients: phosphate sensor commercialization and ammonium sensor development".

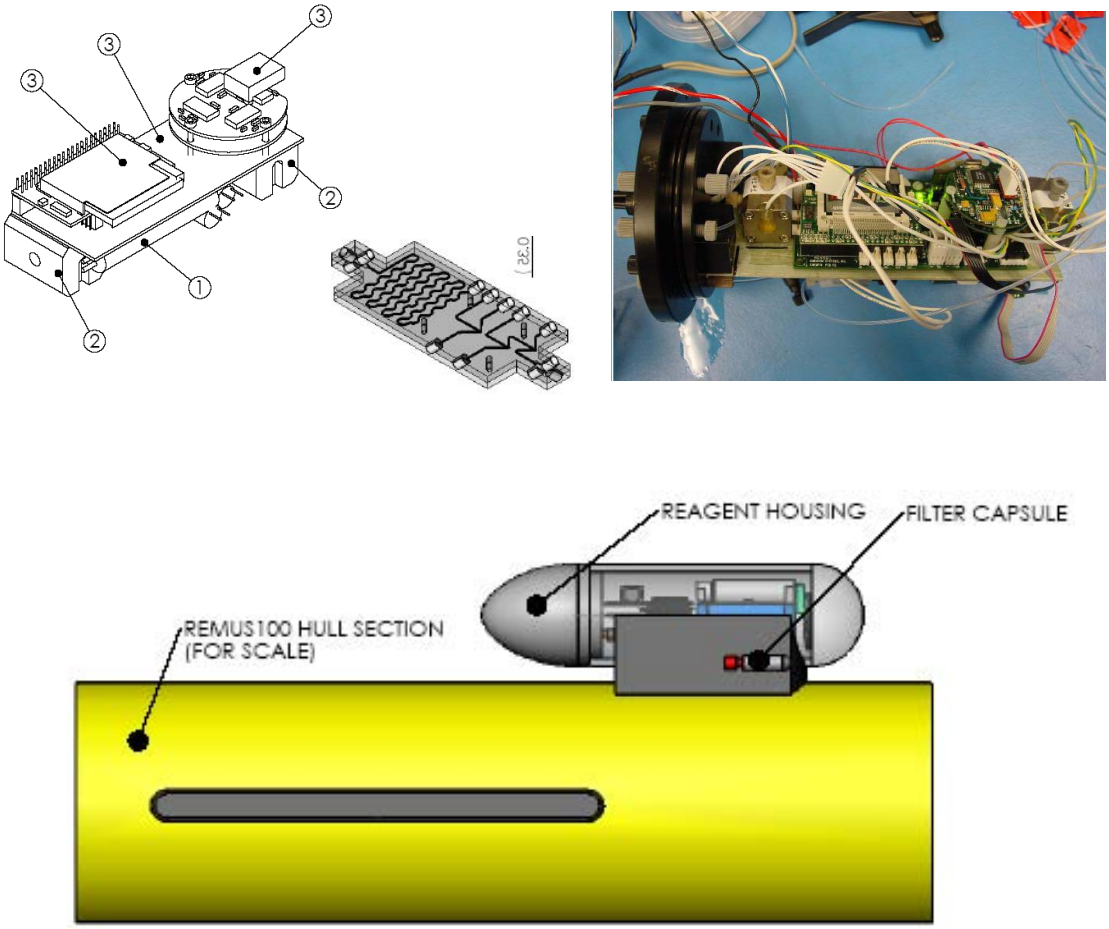

Figure 1. SubChem Systems's compact design for ChemFIN™ the next generation chemical sensing payload for AUVs, Gliders and Profilers. The ChemFIN compact microfluidics(1), optical detection(2) and electronics(3) systems (left) and housing (right) externally mounted onto a REMUS vehicle hull section.

[The ChemFIN™ is designed as an independent compact payload containing a micro-fluidic chemical analyzer that minimizes the power and space demands on the AUV platform.]

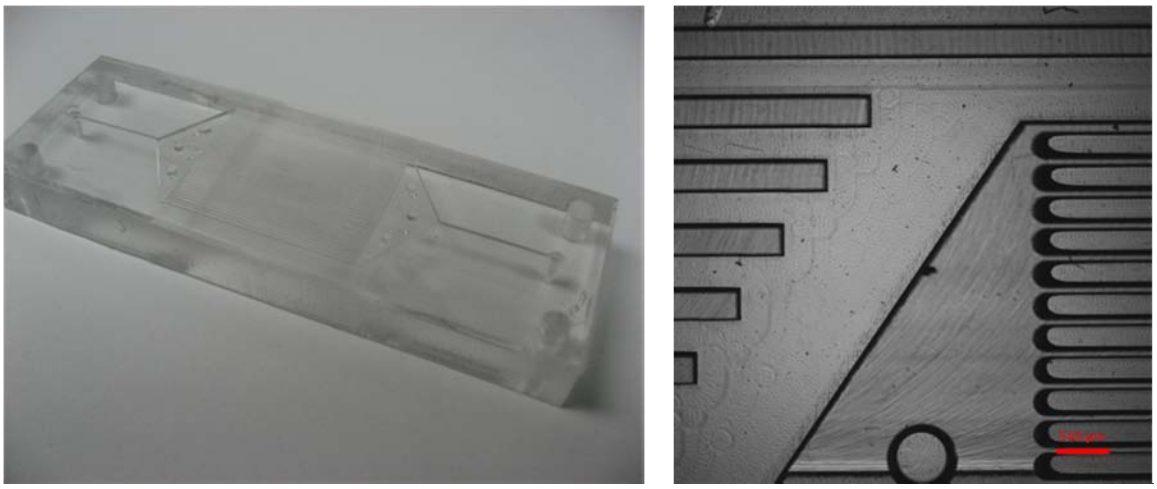

Figure 2. The NRL flow immunosensor coupon consists of a hot-embossed 0.375 in thick PMMA substrate that was tri-solvent bonded to a 0.0625 in thick PMMA coverslip. The footprint of the coupon is 3x1 in^2. The coupon features flat-bottom 0.04 through holes at the inlet and outlet ports.

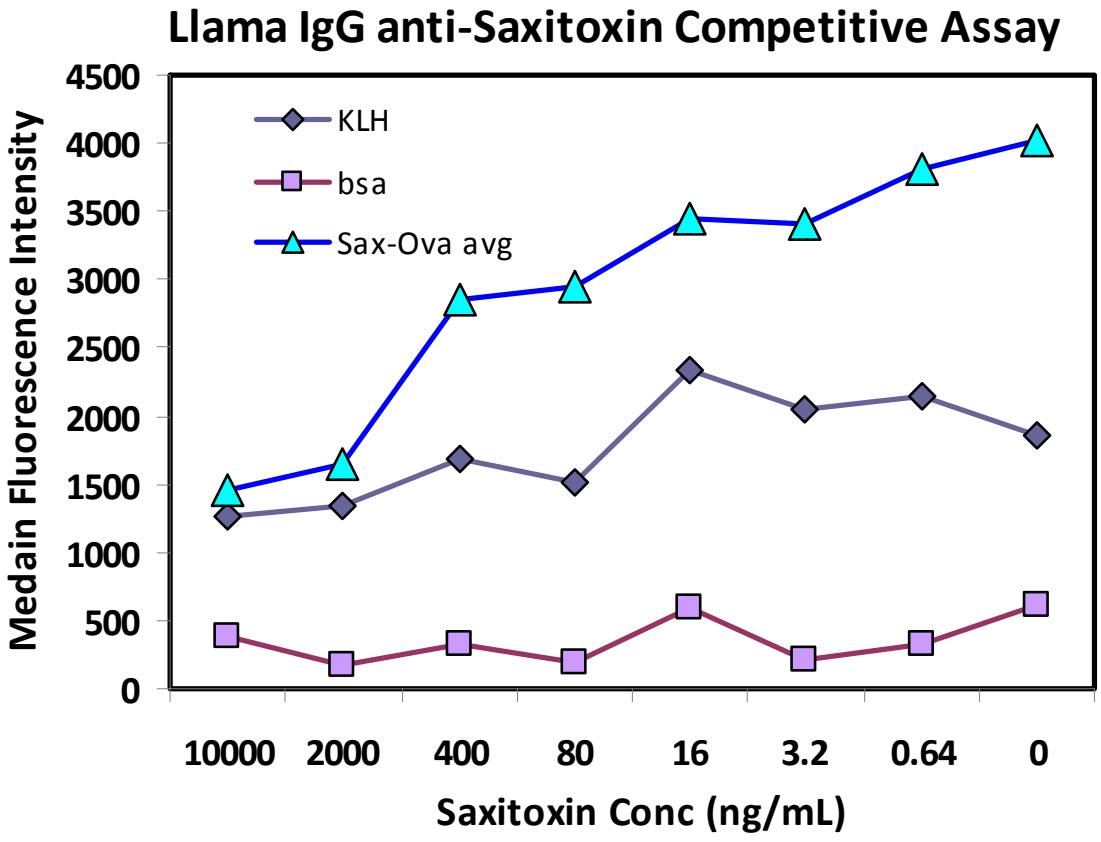

Figure 3. Competitive Binding assay for llama IgG anti-saxitoxins

Figure 4. The "Gen 4" Prototype MarChem Analyzer configured for NRL flow immunosensor capability with the external integrated face sealing coupon holder and the laser-based fluorescence detection of the CY-5 fluorophore.

[The MarChem housing is specifically designed as a compact sensor payload for the REMUS AUV.]